

**Orchestrated downregulation of genes involved in oxidative metabolic pathways in obese vs. lean high-fat young male consumers**

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**Running title:**

Differential gene expression in obesity

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## **Abstract**

There are major variations in the susceptibility to weight gain among individuals under similar external influences (decreased physical activity and excessive calorie intake), depending on the genetic background. In the present study, we performed a microarray analysis and RT-PCR validations in order to find out differential gene expression in subcutaneous abdominal adipose tissue from two groups of subjects that despite living in similar environmental conditions such as a habitual high fat dietary intake (energy as fat >40%) and similar moderate physical activity, some of them were successfully “resistant” (lean) to weight gain, while others were “susceptible” to fat deposition (obese). The classification of up- and down- regulated genes into different categories together with the analysis of the altered biochemical pathways, revealed a coordinated downregulation of catabolic pathways operating in the mitochondria: fatty acid  $\beta$  oxidation ( $P=0.008$ ), TCA cycle ( $P=0.001$ ) and electron transport chain ( $P=0.012$ ). At the same time, glucose metabolism ( $P=0.010$ ) and fatty acid biosynthesis ( $P=0.011$ ) pathways were also downregulated in obese compared to lean subjects. In conclusion, our data showed an orchestrated downregulation of nuclear-encoded mitochondrial gene expression. These genes are involved in cellular respiration and oxidative metabolism pathways, and could play a role in the susceptibility to weight gain in some individuals.

**Keywords:** Obesity; Genetic susceptibility; High fat diet; Metabolic pathways; Microarray.

## **Introduction**

Obesity is a major health problem in the developed world and an increasing problem in low income countries (38). Obesity is strongly associated with different illnesses such as type 2 diabetes and cardiovascular diseases (14, 23). Increasing prevalence of obesity has been proposed to be a consequence of both increased energy availability and reduced energy expenditure superimposed on a genetic background evolved for survival in conditions of food deprivation (30). A number of studies have demonstrated that white adipose tissue (WAT) is a very active endocrine organ, which secretes important molecules involved in the regulation of body weight and insulin sensitivity (1, 17). Moreover, it has been reported the important contribution of WAT to the control of energy homeostasis with the study of tissue-specific knockout models and the discovery of adipocyte-specific secreted factors that have powerful effects on fuel metabolism (6, 39).

Excess of dietary fat has been implicated in the development of obesity and diabetes (5, 35). However, there are large interindividual differences in this compensatory response to increased fat intake. A recent study has evidenced that major changes in the genome of individual animals may be established prior to the introduction of a high-fat diet (19). In a previous study, we recruited two groups of subjects who lived in similar conditions of habitual high-fat dietary intake and moderate physical activity (during at least the 3 last years). Despite living in a condition of chronic disturbance of metabolic homeostasis, the group of lean volunteers was successfully 'resistant' (lean) to weight gain (25), as previously shown in rats (33).

Given the fact that current nutritional habits and lifestyles of most modern humans heavily favour metabolic overload with diminishing physical activity, the goal of this study was focused on unveiling the involvement of specific WAT genes related to

energy utilization in excessive weight gain in presumably predisposed subjects. For this purpose, we applied high density oligonucleotide arrays, a powerful tool (22, 29) to identify and compare patterns of genes differentially expressed in subcutaneous abdominal adipose tissue (WAT) from these two groups of subjects with different susceptibility to weight gain when consuming high-fat diets.

## **Subjects and methods**

### **Study design and Subjects**

Nine lean and nine obese high fat consumers were recruited (range, 22-33 years old for lean and 21-35 years old for the obese group), as previously described, using a validated questionnaire based on self-reported questions about lifestyle and food frequency consumption (25). All subjects were healthy male, non-diabetic, non-hyperlipidemic, taking no oral medications and with a stable body weight during at least the previous 3 months. In order to confirm that the amount and composition of energy ingested was >40% from fat, each subject completed a 3 day weighed food record for two weekdays and one weekend day. The food records were analysed with a computerized program (Medisystem, SanoCare, Madrid, Spain) by a trained nutritionist. To quantify the level of physical activity (PA), each participant completed a validated questionnaire (27) based on self reported questions about their leisure time and their work-time physical activities on a typical work day and on a typical weekend day. In addition, sedentary lifestyle was assessed through the number of hours spent sitting down per week (watching TV or videos, reading or listening to music, etc.) on a typical work day and on a typical weekend day. All the volunteers remained living in the same conditions during at least the last three years.

### **Anthropometric measurements and adipose tissue biopsy**

On the experimental day, volunteers arrived at the Clinica Universidad de Navarra after 12 hours of overnight fast. Anthropometric measurements were made by standard procedures as previously described (25). Then, biopsies (1-2 grams) of subcutaneous abdominal adipose tissue (WAT) were performed by liposuction under local anaesthesia. The samples were washed and soaked in RNA-later (Qiagen, Valencia, California) to avoid RNA degradation and then stored at -80 °C until utilization. The protocol was approved by the Ethical Committee of the University of Navarra at the Clinica Universidad de Navarra, and all subjects gave their written informed consent before participating in the study.

### **RNA preparation**

Total RNA was isolated from each human WAT sample (including adipocytes and stroma-vascular fraction) by homogenization with an ultra-turrax® T 25 basic using TRIzol (Life Technologies, Gaithersburg, MD) according to the manufacturer's instructions and incubated with RNase-free DNase (Ambion, Austin, TX) for 30 min at 37°C. RNA concentration was measured spectrophotometrically and its quality was verified by ethidium bromide staining after agarose gel electrophoresis.

### **Microarray experiments and analysis**

RNA was pooled to minimize the biological variation between the individual lean and obese subjects. Thus, 15 micrograms of total RNA from two pools of 3 lean subjects each (L1 and L2) and two other from 3 obese individuals each (O1 and O2) were used in the standard protocol from Affymetrix to label targets. These targets (biotinylated complementary RNA) were hybridized to the Human HG-U133 A GeneChip arrays (Affymetrix; Santa Clara, CA) at Progenika Biopharma Inc (Bilbao, Spain), using tools obtained from Affymetrix and according to the manufacturer's protocol (Affymetrix,

Santa Clara, CA). Thus, a total of 4 array hybridizations were performed. The obtained signal values were further analyzed with the Affymetrix microarray suite 5.0. software (MAS 5.0) system to examine obesity-dependent increases and decreases in gene expression. After global scaling, the signal detection, the signal low ratio (SLR) and the different call change were calculated and compared between GeneChips. The comparison was performed for all four combinations: obese vs. lean samples; O1 vs. L1, O1 vs. L2; O2 vs. L1; O2 vs. L2 in a double-cross analysis (concordance analysis). The alteration ratios of the gene expression were represented as means of SLR of the four quotients.

The "change call" criteria of Affymetrix software for several known genes related to obesity matched a call change value of 75% ("increase": leptin; "decrease": adiponectin). These changes were verified by real-time PCR (24). For this reason, as cutoff value, concordance in the different call change of 75% or more was chosen in the indications of "increase" and "decrease" for changes in gene expression, as previously described (7).

### **Identification of altered pathways**

The ultimate goal of identifying genes that were differentially expressed in obese compared to lean high fat intakers was to determine whether any of these genes appeared to be part of the same pathway. The approach taken was to use GARBAN software (26, 36) to classify the differentially expressed genes according to KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO biological process criteria. Furthermore, an enrichment analysis was performed using the WebGestalt system (40), which uses the hypergeometric test to identify those pathways in which the number of identified genes exceeded the number expected ( $P < 0.05$ ); Up and downregulated genes were analyzed separately.

### **Real-time PCR analysis**

Differential gene expression was further confirmed by real-time PCR of a subset of genes in individual samples (n=9 in each group). Reagents for real-time PCR analysis of ACADM, ALDH1L1, ETFDH, HADHSC, FASN, SCD and 18S (Assays-on-Demand, TaqMan Reverse Transcriptase reagents, and TaqMan Universal PCR Master mix) were purchased from Applied Biosystems (Foster City, CA) and the conditions were used according to the manufacturer's protocol. Amplification and detection of specific products were performed with the ABI PRISM 7000HT system (Applied Biosystems). Human 18S was used as reference to normalize the expression levels between samples allowing data to be expressed relative to 18S rRNA, therefore compensating for any differences in reverse transcriptase efficacy, as previously described (24).

### **Statistical analysis.**

Data are expressed as means  $\pm$  SE (n=9 in each group). Differences between the lean and obese groups were analysed by the unpaired Student's t test or U of Mann Withney after testing the normality with the Kolmogorov–Smirnov and Shapiro–Wilk tests. A P value  $<0.05$  was considered statistically significant. The SPSS 14.0 version for WINDOWS (SPSS, Chicago, IL, USA) was used for the statistical analysis.

## **RESULTS**

### **Subjects characteristics**

The goal of this study was to compare the gene expression pattern of two groups of subjects with similar habitual fat intake and moderate physical activity but with different susceptibility to weight gain. In fact, despite their habitual high fat intake during the last three years, a group of volunteers remained lean (BMI  $23.1 \pm 0.4$  kg/m<sup>2</sup>) so it was assumed that lean subjects were “resistant” to weight gain. Analysis of three-

day weighed food records (Table 1) revealed that as designed, both groups (lean and obese) had a similar eating pattern with respect to the calorie intake and the energy distribution of macronutrients (carbohydrate, protein and fat). Thus, both groups reported a high percentage of fat in the diet (>40% of energy), with the same proportion of different types of dietary fat (saturated, monounsaturated and polyunsaturated). In addition, both groups reported no differences in the estimation of total METs (Table1).

### **Gene expression profiles of obese compared to lean**

Table 2 and 3 show down-regulated and up-regulated genes involved in metabolic pathways in obese compared to lean subjects, respectively. The enrichment analysis, showed a downregulation of three catabolic pathways operating in the mitochondria, concerning fatty acid oxidation (P=0.008), tricarboxylic acid (TCA) cycle (P=0.001) and electron transport chain (P=0.012) which are closely related to the oxidative phosphorylation processes. In addition, we also found that genes regulating fatty acid biosynthesis (P=0.011) and glucose metabolism (P= 0.010) pathways were also downregulated in obese compared to lean subjects.

#### ***Fatty acid $\beta$ -oxidation cycle***

Our microarray data showed that transcripts for three of the six standard enzymes of the  $\beta$ -oxidation cycle were downregulated in obese subjects: medium chain acyl-CoA dehydrogenases (ACADM), short chain enoyl-CoA hydratase (ECHS1), and short chain 3-hydroxyacyl-CoA dehydrogenase (HADHSC). The downregulation of gene expression of ACADM and HADHSC was also verified by real-time PCR (Fig. 1A).

#### ***TCA cycle***

Three transcripts encoding enzymes of the TCA cycle were downregulated in obese-susceptible subjects: 1) citrate synthase (CS), one of the key regulatory enzymes that catalyze the condensation of oxaloacetate and acetyl coenzyme A to form citrate; 2) the



$\beta$  subunit of the succinyl-CoA synthetase, ADP forming (SUCLA2) and 3)  $\beta$  subunit succinate-CoA ligase, GDP-forming (SUCLG2), two enzymes important to the metabolism of branched chain amino acids.

### ***Glyceroneogenesis***

Surprisingly, both the microarray data and the real-time PCR assessment ascertained that the expression of phosphoenolpyruvate carboxykinase (PCK1 also known as PEPCK), one of the important enzymes for glyceroneogenesis (GNG), was dramatically decreased in obese-susceptible subjects (Table 3; Figure 1B). Furthermore, the gene MCT2 encoding a high affinity pyruvate transporter (21) was also downregulated in obese compared to lean subjects.

### ***Electron transport and Oxidative phosphorylation***

Interestingly, our data showed that the expression of multiple components of the mitochondrial respiratory chain was reduced in obese individuals, including the electron transfer flavoprotein-ubiquinone oxidoreductase (ETFDH), which transfers the electrons generated by the  $\beta$ -oxidation cycle to complex II of the oxidative phosphorylation system (OXPHOS), and the ALDH1L1 that catalyzes this reaction (Table 3; Figure 1A). The expression levels of UQCRC2 (a complex III component) and COX7C (a complex IV component), which catalyzes the electron transfer from reduced cytochrome c to oxygen were also reduced in obese subjects. Furthermore, the gene encoding F1-ATPase assembly protein (ATPAF1) in human mitochondria was also downmodulated in obese subjects (Table 3).

### ***Fatty acid biosynthesis***

Several transcripts encoding for lipogenic enzymes were downregulated in obese subjects, including the transcript for ATP-citrate lyase (ACLY), the acetyl-Coenzyme A carboxylase beta (ACACB), the fatty acid synthase (FASN) and the stearyl-CoA

desaturase 1 (SCD1). The down-regulation of FASN and SCD gene expression was also determined by real-time PCR (Fig. 1B)

### ***Glycolytic pathway***

The gene expression of critical regulators of glucose catabolism was downregulated in obese compared to lean subjects. Indeed, transcript levels for three out of eight glycolytic key enzymes were decreased in obese-susceptible subjects: 1) hexokinase 2 (HK2), which encodes the enzyme that phosphorylates glucose to produce glucose-6-phosphate, committing glucose to the glycolytic pathway; 2) fructose-2, 6-bisphosphatase (PFKFB3), which encodes for an enzyme that is a potent activator of phosphofructokinase, a rate-limiting enzyme for glycolysis, and 3) glutamine-fructose-6-phosphate transaminase 1 (GFPT1), the product of which is the first and rate-limiting enzyme of the hexosamine biosynthesis pathway (HBP).

### ***Amino acid (AA) degradation pathways***

Obesity susceptibility was also associated with a decreased expression of four transcripts coding for enzymes involved in AA degradation: 1) spermidine/spermine acetyltransferase 1 (SAT1), a rate-limiting enzyme in the catabolic pathway of polyamine metabolism 2) methylmalonyl CoA mutase (MUT) a transcript of the valine, leucine, and isoleucine degradation pathway; 3) one transcript encoding for glutamate-ammonia ligase (GLUL), and 4) the aldehyde dehydrogenase 6 family, member A1 (ALDH6A1), which encodes a enzyme that plays a role in the valine and pyrimidine catabolic pathways (Table 3).

## **DISCUSSION**

In a previous study of our group, two groups of subjects with different susceptibility to high-fat induced weight gain were identified (25). In fact, despite having similar

habitual energy intake (with high proportion of energy as fat >40%) and similar patterns of physical activity, some volunteers were successfully “resistant” to weight gain. We hypothesised that the metabolic and hormonal mechanisms underlying the resistance to weight gain were probably genetically determined. As expected, the microarray analysis revealed a differential gene expression pattern in WAT of obese and lean high-fat consumers. The classification of genes differentially expressed in obese compared to lean subjects revealed an orchestrated downregulation of catabolic pathways operating in the mitochondria (fatty acid oxidation, TCA cycle and electron transport), with catabolic (glycolysis) and anabolic (glyceroneogenesis and lipogenesis) pathways of the cytoplasm.

Interestingly, our data revealed that several transcripts encoding for key enzymes in the  $\beta$ -oxidation cycle and that multiple components of the mitochondrial respiratory chain were downregulated in obese subjects. Heilbronn et al (13) have demonstrated that different biomarkers of mitochondrial biogenesis and metabolism are reduced in overweight and obese insulin-resistant subjects. Two other studies using cDNA microarrays have reported that mitochondrial metabolism in both muscle and adipocytes is disturbed in subjects with insulin resistance, type 2 diabetes and even in subjects with family history for diabetes (28, 32). Both trials found a decrease in the expression of a subset of genes involved in mitochondrial oxidative metabolism, suggesting that impaired regulation of mitochondrial function could be an important mechanism linked to obesity and the metabolic syndrome. On the other hand, our data also showed that ATPAF1, which was reported as one of the assembly factors for the F1-ATPase in human mitochondria (37), was also downmodulated in obese subjects. Therefore, all the observed changes suggest that a decrease in both mitochondrial oxidative activity and mitochondrial adenosin triphosphate (ATP) synthesis could underlie the susceptibility to

HFD-induced obesity. This hypothesis is in agreement with the results obtained by Hao et al (12), which showed that mice with deleted PAS kinase (a cellular metabolic sensor) are resistant to high fat diet-induced obesity and insulin resistance, partly due to an increase in the rate of oxidative metabolism and ATP production. This finding could explain why after a high fat load of 50% of their energy requirements, obese-susceptible subjects were not able to increase their rates of fat oxidation more than the lean subjects, despite their higher metabolic mass (25). The previously reported similar rates of postprandial fat oxidation, together with the observed downregulation of genes involved in mitochondrial fatty acid oxidation and electron transport pathways suggest that obese-susceptible individuals may have a difficulty to properly adjust the amount of fat intake to the amount of fat oxidized. Similarly, another study, has observed an impaired capacity to regulate fat oxidation in the obese-insulin resistant state (4). Recently, the NUGENOB study has shown the importance of obesity-related polymorphisms in this area (8).

Both theoretical aspect and experimental data from research on mitochondria indicate that cytosolic metabolism should be coordinated with mitochondrial metabolism so that both are oscillating in synchrony to permit the most efficient utilization of substrates resulting in energy production (ATP/ADP and NAD/NADH ratios) (11). In fact, glycolysis pathway generates cytoplasmic NADH, which is regenerated to NAD by the glycerol phosphate shuttle. Then, the electrons produced flow to Coenzyme Q, which has been shown by microarray to be downregulated in our obese-susceptible subjects. It can be speculated that this observation could explain the downregulation of transcripts encoding for enzymes of glycolysis pathway such as PFKFB3, which is highly expressed in adipose tissue and seems to exert a function that contributes to adiposity, but, on the other hand, protects against the inflammatory response of adipose tissue and the insulin

resistance induced by diet (16). Thus, the downregulation in gene transcripts involved in the glycolytic pathway in obese subjects is in agreement with and could explain the lower postprandial carbohydrate oxidation that we observed in these obese-susceptible subjects (25).

In vitro studies demonstrated that a decrease in mitochondrial ATP production resulted in the inhibition of FA synthesis (34). These data were corroborated by Kerner and Hopel (18), who demonstrated that cytosolic fatty acid synthesis and mitochondrial  $\beta$ -oxidation are the two pathways reciprocally regulated through the inhibitory effect of malonyl-CoA on CPT-1 activity. Recently, another investigation has proved that saturated fatty acids (like palmitic and stearic) are able of disrupting the mitochondrial function reducing ATP generation and the hyperpolarization of the mitochondria (15). Such data could explain the downregulation of key mediators of the fatty acid de novo biosynthesis pathway (FASN, SCD) in obese-susceptible subjects, which utilize the acetyl-CoA produced by the  $\beta$ -oxidation cycle to store energy for later use. As previously described, subjects with established obesity have an increased hepatic lipogenesis, but decreased lipogenic activity in adipose tissue (10). It has been suggested that the dysregulation of glyceroneogenesis (GNG) in WAT is a causal factor for obesity (3) and that a reduction in the expression of PCK1 (a key enzyme for GNG) and G6P, improved insulin sensitivity and glucose metabolism in mice (2). However, our data revealed that PCK1 was dramatically decreased in obese-susceptible subjects. This apparently surprising result is in agreement with a publication which shows that GNG in human SCAAT decreases with BMI in overweight women, suggesting that the decreased GNG in overweight people could participate in the metabolic syndrome appearance (20), in contrast, with another study which found that the expression of PCK1 mRNA was positively correlated with body fat in nondiabetic women (9). These

data imply that more research is needed in this area. Furthermore, in a previous study, we reported that obese-susceptible subjects exhibits a lower expression of the AQP7 gene, encoding an aquaglyceroprotein, which could be implicated in the susceptibility to obesity by reducing glycerol release and promoting the accumulation of lipids in the adipose tissue (24). Therefore, the accumulation of glycerol due to the downregulation of AQP7 could be involved in the downregulation of PCK1 observed in obese-susceptible subjects.

Our data suggest that the differences in the metabolic adaptation to a high-fat diet consumption between lean and obese subjects could be the result of a coordinated down-regulation in the expression of mitochondrial genes encoding key proteins involved in fat oxidation and ATP production, and cytosolic genes involved in lipolytic and glycolytic pathways. This is consistent with the Neel's thrifty genotype hypothesis, suggesting that in subjects genetically predisposed to obesity, a sustained increase in the high fat and energy intake may trigger biological actions leading to increase the efficiency of energy storage by limiting fuel oxidation and ATP production (31), by probably decreasing the expression of nuclear-encoded mitochondrial genes involved in oxidative metabolism. However, with the current data, it cannot be totally ruled out if the downregulation of these genes involved in oxidative metabolic pathways in obese subjects is a cause or a consequence of obesity, and more studies are needed to shed some light on this area.

In summary, the higher expression levels in genes involved in oxidative metabolism in lean subjects as compared to obese, could contribute to their genetically mediated resistance to the development of obesity by a high fat diet suggesting a novel alternative therapeutical approach to be investigated.

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**Table 1.** Body composition, metabolic rate, dietary assessment and physical activity pattern of lean-resistant and obese-susceptible subjects participating in the study.

	<b>Lean (n=9)</b>	<b>Obese (n=9)</b>	<b>P</b>
BMI	23.1±0.4	34.7±1.2	0.000
Body fat (%)	14.4 ± 0.9	29.8 ± 1.2	0.000
Total Energy intake (Kj/day)	11564±1081	11700±715	0.918
MUFA (E%)	26.9±1.3	25.5±1.7	0.554
SFA (E%)	12.1±0.9	10.4±0.5	0.124
PUFA (E%)	4.9±0.6	6.3±1.4	0.361
Complex CHO (E%)	17.4±1.9	18.4±2.1	0.722
Simple CHO (E%)	16.9±1.6	14.1±2.1	0.312
Protein (E%)	16.2±1.2	18.1±0.8	0.211
RMR (KJ/min)	5.03 ± 0.12	6.18 ± 0.14	0.000
Total activity (METs h/week)	146.0±10.4	168.9±10.1	0.141

%E, percentage of energy; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; CHO, carbohydrate; RMR, resting metabolic rate; METs-h/wk, metabolic equivalents-hour per week, represent the ratio of energy expended during each specific activity to resting metabolic rate and are independent of body weight.

**Table 2.** Upregulated transcripts involved in metabolic pathways in subcutaneous abdominal adipose tissue (SCAAT) of obese-susceptible compared to lean-resistant subjects based on KEGG database.

	Gene Symbol	SLR	SD	Ref seq	E.C n°
<b>Lipid Metabolism</b>					
Arachidonic acid metabolism	AKR1C3	0.88	0.32	NM_003739	1.1.1.188
<b>Metabolism of Cofactors and Vitamins</b>					
Riboflavin metabolism	ACP5	1.58	0.53	NM_001611	3.1.3.2
<b>Iron ion metabolism</b>					
	FTL <sup>(1)</sup>	0.53	0.17	NM_000146	
	TFRC <sup>(1)</sup>	0.75	0.33	NM_003234	

SLR: Signal Log Ratio; SD: Standard deviation; EC: Enzyme compound; <sup>(1)</sup> classified by GO biological function criteria. **AKR1C3: Aldo-keto reductase family 1 member C3. ACP5: acid phosphatase 5, tartrate resistant. FTL: Ferritin light chain; TFRC, transferrin receptor (p90, CD71).**

**Table 3.** Downregulated transcripts involved in metabolic pathways in subcutaneous abdominal adipose tissue (SCAAT) of obese-susceptible compared to lean-resistant subjects based on KEGG database.

Gene name	Gene Symbol	SLR	SD	Ref seq	E.C
<b>Lipid Metabolism</b>					
Fatty acid biosynthesis					
Stearoyl-CoA desaturase-1	SCD1	-0.83	0.22	NM_005063	1.14.99.5
Acetyl-CoA carboxylase $\beta$	ACACB	-0.43	0.53	NM_001093	6.4.1.2
Fatty acid synthase	FASN	-1.00	0.62	NM_012347	2.3.1.85
Fatty acid $\beta$ oxidation					
Alcohol dehydrogenase 1B	ADH1B	-1.15	0.50	M21692	1.1.1.1
Medium chain acyl-CoA dehydrogenase	ACADM	-0.80	0.12	NM_000016	1.3.99.3
Long chain-fatty-acid-CoA ligase 1	ACSL1	-0.63	0.22	NM_001995	6.2.1.3
Short chain enoyl-CoA hydratase, , 1	ECHS1	-0.48	0.26	NM_004092	4.2.1.17
3-hydroxyacyl-CoA dehydrogenase	HADHSC	-1.00	0.44	NM_005327	1.1.1.35
<b>Citrate cycle (TCA cycle)</b>					
$\beta$ subunit of the succinyl-CoA synthetase, ADP-forming	SUCLA2	-0.50	0.37	NM_003850	6.2.1.5
$\beta$ subunit succinate-CoA ligase, GDP-forming	SUCLG2	-0.38	0.38	NM_003848	6.2.1.4
Citrate synthase	CS	-0.68	0.26	NM_004077	2.3.3.1
ATP-citrate lyase	ACLY	-0.35	0.10	NM_001096	2.3.3.8
Phosphoenolpyruvate carboxykinase 1 (also PEPCK)	PCK1	-1.78	0.73	NM_002591	4.1.1.32
Monocarboxylate Transporter 2	MCT2 <sup>(2)</sup>	-0.70	0.29	AF049608	
<b>Electron transport and Oxidative Phosphorylation Pathways</b>					
Electron transfer flavoprotein-ubiquinone oxidoreductase	ETFDH	-0.95	0.25	NM_004453	1.5.5.1
Aldehyde dehydrogenase 1 family, member L1	ALDH1L1 <sup>(1)</sup>	-1.15	0.85	NM_012190	1.5.1.6
Cytochrome c oxidase subunit 7C	COX7C	-0.72	0.22	NM_001867	1.9.3.1
Cytochromes $b_5$	CYB5 <sup>(1)</sup>	-1.35	0.62	M22976	
Cytochrome P450 26B1	CYP26B1 <sup>(1)</sup>	-0.40	0.26	NM_019885	
Cytochrome b-c1 complex subunit 2	UQCRC2	-0.45	0.19	NM_003366	
F1-ATPase assembly protein factor 1	ATPAF1 <sup>(2)</sup>	-0.60	0.58	NM_022745	1.10.2.2
<b>Carbohydrate Metabolism</b>					
Glycolysis					
Fructose-2, 6-bisphosphatase	PFKFB3	-0.93	0.51	NM_004566	3.1.3.11
Hexokinase 2	HK2	-0.83	0.79	NM_000189	2.7.1.1
Aminosugars metabolism					
Glutamine-fructose-6-phosphate transaminase 1	GFPT1	-0.38	0.05	NM_002056	2.6.1.16
Pentose and glucuronate interconversions					
Dicarbonyl/L-xylulose reductase	DCXR	-0.38	0.05	NM_016286	1.1.1.10
<b>Amino Acid Metabolism</b>					
Arginine and proline metabolism					
Spermidine/spermine acetyltransferase 1	SAT1	-0.55	0.10	NM_002970	2.3.1.57
High-mobility group box 2	HMGB2	-0.28	0.17	NM_002129	4.1.1.50
Glutamate metabolism					
Glutamate-ammonia ligase (also GS)	GLUL	-0.68	0.05	AL161952	6.3.1.2
Valine, leucine and isoleucine degradation					
Aldehyde dehydrogenase 6 family, member A1	ALDH6A1	-0.68	0.59	NM_005589	1.2.1.27
Methylmalonyl CoA mutase	MUT	-0.80	0.73	NM_000255	5.4.99.2
Nitrogen metabolism					
Carbonic anhydrase III	CA3	-2.05	0.77	NM_005181	4.2.1.1
<b>Metabolism of Cofactors and Vitamins</b>					
Pantothenate and CoA biosynthesis					
Dihydropyrimidine dehydrogenase	DPYD	-0.35	0.10	NM_000110	1.3.1.2
<b>Iron ion metabolism<sup>(2)</sup></b>					
Iron transport					
Transferrin	TF <sup>(1)</sup>	-1.35	0.86	NM_001063	

SLR: Signal Log Ratio; SD: Standard Deviation; EC: Enzyme compound ; <sup>(1)</sup>classified by GO biological

function criteria; <sup>(2)</sup>classified by findings in literature.

## Legends to the figures

**Fig. 1.** mRNA expression by TaqMan real-time quantitative PCR of differentially expressed genes identified by microarray. A) Genes encoding key enzymes in  $\beta$ -oxidation and electron transport. B) Genes encoding enzymes in Lipogenesis and TCA pathways in SCAAT adipose tissue of lean and obese high fat intakers; n=9 in each group. Data (means  $\pm$  SEM) were calculated by the  $2^{-\Delta\Delta C_t}$  method (mean value for lean subjects was set at 1) and were analyzed at  $\Delta C_t$  stage to exclude potential bias because of averaging data that had been transformed through the equation  $2^{-\Delta\Delta C_t}$ . Human18S rRNA was used as reference to normalize the expression levels. \*  $P < 0.05$ ; \*\* $P < 0.01$  lean  $\square$ , vs. obese  $\blacksquare$  subjects. **ACADM:** medium chain acyl-CoA dehydrogenase. **HADHSC:** 3-hydroxyacyl-CoA dehydrogenase. **ALDH1L1:** aldehyde dehydrogenase 1 family, member L1. **ETFDH:** Electron transfer flavoprotein-ubiquinone oxidoreductase. **FASN:** Fatty acid synthase. **SCD1:** Stearoyl-CoA desaturase-1. **PCK1:** Phosphoenolpyruvate carboxykinase 1.

**Fig. 2.** Schematic representation of the main energy metabolic pathways that may lead to obesity. Enzymes/Receptors in boxes are those that are encoded by genes differentially regulated in obese-susceptible compared to lean-resistant subjects (see text for references); \*mRNA expression was verified by real-time PCR.

The results of the microarrays show that the differences in the metabolic adaptation to a high fat diet between resistant-lean and obese-susceptible subjects could be the result of a coordinated down-regulation in the expression of mitochondrial genes encoding key proteins involved in fat oxidation and ATP production. As both, cytosolic and mitochondrial metabolisms are coordinated oscillating in synchrony resulting in energy production of the ATP/ADP and NAD/NADH ratios for the required reducing potential. The downregulation of the electron transport pathway will lead to the downregulation of

cytosolic genes involved in lipolytic and glycolytic pathways. Furthermore, adipose tissue seems to trigger defense mechanisms against fat deposition by downregulating glyceroneogenesis and lipogenesis pathways. Abbreviations: G, Glucose; F, Fructose; P, phosphate; GAP, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; G3P, glycerol-3-phosphate; PEP, phosphoenolpyruvate; OAA, oxalacetate; A-CoA, Acyl-CoA; T- $\Delta^2$ -E-CoA, Trans  $\Delta^2$ -Enoil-CoA; 3-L-OH-A-CoA, 3-L-Hydroxy-Acyl-CoA; 3-K.A-CoA, 3-Ketoacyl-CoA.